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Biotechnological Potential of *Weizmannia ginsengihumi* in the Conversion of Xylose into Lactic Acid: A Sustainable Strategy

Larissa Provasi Santos ¹, Ingrid Yoshimura ¹ , Fernanda Batista de Andrade ¹ and Jonas Contiero ^{1,2,*} 

¹ Department of General and Applied Biology, Institute of Biosciences, São Paulo State University (Unesp), Rio Claro CEP 13506-900, SP, Brazil; larissa.provasi@unesp.br (L.P.S.); ingrid.yoshimura@unesp.br (I.Y.); andradefb.biomol@gmail.com (F.B.d.A.)

² Institute of Research in Bioenergy, São Paulo State University (Unesp), Rio Claro CEP 13500-230, SP, Brazil

* Correspondence: jonas.contiero@unesp.br; Tel.: +55-19-3526-4149

Abstract

The aim of this study was to isolate *Weizmannia* spp. that produce lactic acid from xylose and use an experimental design to optimize the production of the metabolite. After isolation, the experiments were conducted in xylose-yeast extract-peptone medium. The identification of isolates was performed using the 16S rDNA PCR technique, followed by sequencing. A central composite rotatable design (CCRD) was used to optimize the concentrations of the carbon source (xylose), nitrogen source (yeast extract and peptone), and sodium acetate. Two strains were considered promising for lactic acid production, with *W. coagulans* BLMI achieving greater lactic acid production under anaerobic conditions ($21.93 \pm 0.9 \text{ g} \cdot \text{L}^{-1}$) and a yield of 69.18%, while the strain *W. ginsengihumi* BMI was able to produce $19.79 \pm 0.8 \text{ g} \cdot \text{L}^{-1}$, with a yield of 70.46%. CCRD was used with the *W. ginsengihumi* strain due to the lack of records in the literature on its use for lactic acid production. The carbon and nitrogen sources influenced the response, but the interactions of the variables were nonsignificant ($p < 0.05$). The response surface analysis indicated that the optimal concentrations of carbon and nitrogen sources were 32.5 and 3.0 $\text{g} \cdot \text{L}^{-1}$, respectively, without the need to add sodium acetate to the culture medium, leading to the production of $20.02 \pm 0.19 \text{ g} \cdot \text{L}^{-1}$, productivity of 0.55 $\text{g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ after 36 h of fermentation, and a residual sugar concentration of $12.59 \pm 0.51 \text{ g} \cdot \text{L}^{-1}$. These results demonstrate the potential of *W. ginsengihumi* BMI for the production of lactic acid by xylose fermentation since it is carried out at 50 °C, indicating a path for future studies

Keywords: pentose; organic acids; fermentation; experimental design; *Weizmannia ginsengihumi*



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1. Introduction

Lactic acid is characterized by the presence of three distinct functional groups. One terminal carbon atom forms a carboxylic acid group, while the other is part of a hydrocarbon chain. The central carbon atom carries a hydroxyl group, giving it the properties of an alcohol. The notable properties of lactic acid include its solubility in water and water-miscible organic solvents, low volatility, with a boiling point of 82 °C at 0.5 mm Hg, and a molar mass of 90.08 g [1]. The enantiomeric forms, L(+) lactic acid and D(−) lactic acid, have identical chemical and physical properties as the pure form [2]. L(+) lactic acid is considered an endogenous compound, such as L-lactate found in human blood, while D(−) lactic acid is less involved in basic metabolic processes, except in lactic acid bacteria [3,4]. The US Food and Drug Administration classifies it as generally recognized as safe (GRAS), enabling its use in the food, chemical, pharmaceutical, and cosmetic industries [5].

Lactic acid serves as a monomer in the synthesis of polylactic acid, which is a biodegradable polymer used in the production of food packaging, mulching film, garbage bags, and protective clothing [6]. Lactic acid and its polylactic acid derivatives stand out as one of the most important markets in the chemical industry [7]. In 2022, the global lactic acid market was estimated at around USD 3.1 billion, with an expected annual growth rate of 8% between 2023 and 2030 [8]. The increasing demand for lactic acid is driven by factors such as the growing search for environmentally friendly products and the rising prices of petrochemical derivatives, coupled with limited fossil energy reserves [7,9].

The fermentation process for lactic acid production has several advantages over chemical synthesis, such as the use of low-cost substrates, lower temperatures, lower energy consumption, high enantiomeric purity, and lower environmental impacts [10]. However, the biological production of lactic acid can be influenced by several factors, such as temperature, pH, the microorganism employed, and the carbon source [11]. Different types of substrates can be used as a carbon source for microorganism growth. Sugar in its pure form (glucose) stands out as an excellent carbon source because it is free of impurities, facilitating the downstream step. However, high-purity sugars have high costs [12].

Another crucial point is the addition of neutralizing agents to prevent the low pH generated by lactic acid production from exerting a negative impact on microorganism growth. However, including these agents in the medium can imply other factors, such as cell toxicity and the generation of unwanted products, such as plaster when the calcium carbonate is used. Additionally, lactic acid precipitation may occur, requiring an additional step for extraction and making the downstream step increasingly expensive and environmentally harmful [11]. Thus, lactic acid production faces challenges, such as productivity and the high costs involved in the bioprocess in both upstream and downstream stages [13]. Considering the influence of the carbon source as a determinant factor in the cost of lactic acid production, incorporating low-cost substrates, such as agricultural waste products, is a promising option that can mitigate economic challenges and play a crucial role in responsible environmental management. The adoption of this approach not only meets the needs of lactic acid production but also resonates with the principles of the circular economy, adding value to products often considered waste and minimizing the environmental impact associated with improper disposal [14,15].

Sugarcane bagasse, which is rich in pentoses, stands out as an example of waste strategically used as a carbon source for lactic acid production. The 2022/2023 sugarcane crop was estimated at 610 million tons, representing a 5.4% increase compared to the previous crop [16]. This grass is used in the production of sugar and ethanol, generating bagasse as a lignocellulosic waste product. Despite being used as an energy source by mills, bagasse remains the primary waste in agribusiness [17]. Comprising cellulose, hemicellulose, and lignin [18], bagasse contains xylan, which is a heteropolymer composed of pentoses (xylose and arabinose), hexoses, and/or uronic acids [19]. However, pentoses face metabolic challenges. There is a need to find bacteria capable of efficiently converting xylose into lactic acid, especially in environments rich in sugarcane waste, such as sugar and ethanol mills. This approach, which is aligned with the principles of circular economy, not only promotes the sustainable use of waste but also offers an innovative solution to overcome metabolic limitations, boosting efficiency in lactic acid production. The genus *Weizmannia* consists of three species, namely *W. coagulans*, *W. ginsengihumi* and *W. acidiproducens*. These species constituted the Coagulans clade that belonged to the genus *Bacillus*. Thus, it was suggested that 17 clades of *Bacillus* be recognized as new genera, namely *Alteribacter*, *Ectobacillus*, *Evansella*, *Ferdinandcohnia*, *Gottfriedia*, *Heyndrickxia*, *Lederbergia*, *Litchfieldia*, *Margalitia*, *Niallia*, *Priestia*, *Robertmurraya*, *Rossellomorea*, *Schinkia*, *Siminovitchia*, *Sutcliffeiella* and *Weizmannia* [20]. *W. coagulans* is a species that has been

gaining attention due to its advantageous growth characteristics, including the ability to grow at 50–60 °C, simple nutritional requirements, and high optical purity of the L(+) lactic acid enantiomer [21,22]. This elevated temperature range is particularly beneficial, as it reduces the risk of contamination by mesophilic organisms, enabling non-sterile fermentation processes and lowering production costs. Moreover, thermotolerant strains can exhibit enhanced metabolic activity at higher temperatures, potentially increasing lactic acid productivity [23,24]. *W. ginsengihumi* LGHNH (KCTC 14462BP) was investigated to produce indole-3-acetic acid (IAA), a plant growth-promoting hormone (1.38 µg/mL to 2.22 µg/mL) [25]. Tolieng et al. [26] isolated from soils the strains designated PP-18T, JC-4, and JC-7, which, through the results of phenotypic chemotaxonomic characteristics and whole-genome analysis, indicated that the strains should be represented as a novel species within the genus *Weizmannia*, with the name *W. acidilactici* sp. being proposed. The lactic acid production of strains using 120 g/L glucose as substrate resulted in a yield of 0.64 ± 0.06 , 0.63 ± 0.00 , and 0.90 ± 0.01 , respectively, for PP-18T, JC-4, and JC-7. Zhang et al. [27] present a review on *W. coagulans*, where they indicate that it is an ideal probiotic candidate for health with scientific evidence acquired through its administration since it is capable of improving gut health through the regulation of gut microbiota, modulation of immunity, and improving digestibility and metabolism. Through a review, the favorable effect of different strains of *W. coagulans* on animals and humans was evaluated. They show that the functional properties of *W. coagulans* are extensively recognized. In animals, these probiotics can promote nutrient absorption, reduce mortality, and enhance the slaughter rate in livestock and poultry. In humans, it can be used to treat gastrointestinal disorders, immunomodulation, depressive symptoms, and non-alcoholic fatty liver [28].

Therefore, the central objective of this research was to isolate and identify bacteria with the ability to produce lactic acid through the efficient metabolism of xylose. To achieve this purpose, prospecting was performed to obtain microorganisms found in environments containing sugarcane waste, such as sugar and ethanol processing mills. Once promising bacterial strains were identified, the research focused on understanding the factors that influence the lactic acid production bioprocess. For such, an experimental design was employed to optimize cultivation conditions. Optimization efforts were focused on determining the ideal concentrations of carbon, nitrogen, and salt to maximize the production of the target metabolite. This systematic approach not only enhances the efficiency of lactic acid production from xylose but also contributes to a deeper understanding of the key elements governing this bioprocess, thus establishing a foundation for future industrial applications and advances in biotechnology.

2. Materials and Methods

2.1. Isolation and Screening of *Weizmannia* spp. (*Bacillus* sp.) Strains from Sugarcane Bagasse

Microorganisms were isolated from sugarcane bagasse obtained from the Granelli mill located in the municipality of Charqueada, state of São Paulo, Brazil (22°38'75.34" S, 47°54'90.08" W). For isolation, 1.0 g of sugarcane bagasse was suspended in 50 mL of xylose, yeast extract, and peptone (XYP) medium and maintained in a water bath at 80 °C for 10 min. The XYP medium consists of (g·L⁻¹): xylose (20), yeast extract (10), peptone (10), and sodium acetate (10). Additionally, 5 mL·L⁻¹ of a salt solution is added, which contains (g·L⁻¹): MgSO₄·7H₂O (40), MnSO₄·4H₂O (1.6), FeSO₄·7H₂O (2.0), and NaCl (2.0), as modified from Coelho et al. [29]. The flasks were incubated for 24 h at 50 °C, with shaking at 150 rpm, followed by static incubation in a bacteriological oven also at 50 °C. Next, serial dilution was performed in saline solution (0.85%). Subsequently, 100 µL were added to Petri dishes containing XYP agar medium supplemented with calcium carbonate (12 g·L⁻¹). The aliquots were subjected to the spreading technique using a Drigalski loop,

followed by incubation for 48 h at 50 °C. Halo-forming colonies were then selected and purified using the streak plate method in Petri dishes containing XYP agar medium with calcium carbonate, followed by incubation for 48 h at 50 °C. The isolates obtained were stored at −80 °C in cryogenic tubes containing a 1:1 solution of liquid culture medium (LB) and 40% glycerol. The lactic acid concentration was measured as described in the item quantification of lactic acid and residual sugar.

2.2. Identification and Molecular Characterization

DNA extraction was performed using the Illustra Bacteria GenomicPrep Mini Spin kit (GE Healthcare, Chicago, IL, USA), according to the manufacturer's instructions.

The 16S rDNA gene was amplified by polymerase chain reaction (PCR) using the primers 27f (5'-AGAGTTGATCCTGGCTCAG-3') and 1492RT (5'-ACGGCTACCTTGTTACGACTT-3'). Each reaction contained: 1× buffer + 2.5 mM MgCl₂; 0.2 mM dNTPs; 10 pM of each primer; 1.0 U of Taq DNA Polymerase (Invitrogen, Waltham, MA, USA); 5 µL of the DNA sample; in a solution with a final volume of 50 µL. The amplification reaction was performed in the MaxyGene II thermocycler (Axygen, New York, NY, USA), and the quality of the PCR product was validated by agarose gel electrophoresis. The nucleotide sequence of the amplified 16S rDNA gene was determined using an ABI 3500 automated sequencer (Applied Biosystems, Carlsbad, CA, USA). Alignment of the forward and reverse sequences was performed using BioEdit, generating the consensus sequence. Online similarity searches were conducted using BLAST, <https://blast.ncbi.nlm.nih.gov>, accessed on 20 June 2022, provided by the National Center for Biotechnology Information (NCBI). Multiple sequence alignment was performed using BioEdit. Characterizations of the isolated strains, such as Gram staining and growth curve, were conducted in XYP medium at 50 °C and 150 rpm.

2.3. Bacterial Growth Curve

Strains previously stored in cryovials were initially inoculated at a 1:100 ratio into 125 mL Erlenmeyer flasks containing 25 mL of LB medium and incubated for 24 h. After incubation, the optical density (OD) of the cultures was adjusted to 0.1 at 600 nm, and the cultures were transferred to 500 mL Erlenmeyer flasks containing 100 mL of XYP medium. The flasks were incubated at 50 °C with agitation at 150 rpm. Over a 14-h period, 1 mL aliquots were collected every hour to measure bacterial growth by monitoring OD at 600 nm. All experiments were conducted in triplicate.

2.4. Fermentation

The isolated strains stored at −80 °C were activated in Lysogeny Broth (LB) medium composed of (g·L^{−1}) bacteriological peptone (10), yeast extract (5), and sodium chloride (10). The strains were grown for 24 h at 50 °C, with shaking at 150 rpm. The bacteria were then transferred to an inoculum containing XYP medium under the same culture conditions, and maintained according to the growth curve. Fermentation was conducted in 250 mL Erlenmeyer flasks containing 50 mL of XYP medium and 0.1 (OD 600 nm) of the isolates, followed by the addition of calcium carbonate at a concentration of 10 g·L^{−1}. Anaerobic tests were conducted in 250 mL Erlenmeyer flasks containing 50 mL of medium sealed with a silicone stopper, with an opening for nitrogen injection and another opening for pressure adjustment. There were 0.22 µm PTFE filters at both inlets to maintain sterility.

2.5. Central Composite Planning and Response Surface Methodology

To investigate the influence of the concentrations of the carbon source (xylose), nitrogen source (yeast extract and peptone), and sodium acetate, a central composite rotatable design

(CCRD) was planned with four replicates at the central point, totaling 18 experiments. The levels used for coding the independent variables are presented in Table 1.

Table 1. Matrix of CCRD 2³ planning for cultivation conditions in coded and real levels of carbon source (xylose) (X₁), nitrogen source (yeast extract and peptone) (X₂), and sodium acetate (X₃).

Trials	Carbon Source (X ₁)		Nitrogen Source (X ₂)		Sodium Acetate (X ₃)	
	Coded Level	Real Values (g·L ⁻¹)	Coded Level	Real Values (g·L ⁻¹)	Coded Level	Real Values g·L ⁻¹
1	−1	15	−1	1.5	−1	5
2	−1	15	−1	1.5	1	15
3	−1	15	1	4.5	−1	5
4	−1	15	1	4.5	1	15
5	1	50	−1	1.5	−1	5
6	1	50	−1	1.5	1	15
7	1	50	1	4.5	−1	5
8	1	50	1	4.5	1	15
9	0	32.5	0	3	0	10
10	0	32.5	0	3	0	10
11	−1.788	1.19	0	3	0	10
12	1.788	63.8	0	3	0	10
13	0	32.5	−1.788	0.31	0	10
14	0	32.5	1.788	5.68	0	10
15	0	32.5	0	3	−1.788	1.05
16	0	32.5	0	3	1.788	18.94
17	0	32.5	0	3	0	10
18	0	32.5	0	3	0	10

From the responses “lactic acid production”, “bacterial growth”, and “residual sugar,” multiple regression analyses were conducted, generating the regression Equation (1) below:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (1)$$

2.6. Analytical Methods

2.6.1. Determination of Bacterial Growth

For the determination of bacterial biomass, the culture was first centrifuged at 12,000 × g for 10 min at room temperature to obtain the pellet. The resulting pellet was then washed with a 1:1 solution of 0.3 N HCl to remove residual carbonate, followed by vortex agitation and a second centrifugation step under the same conditions. The biomass was then washed with 0.85% saline solution, repeating the vortexing and centrifugation process. The biomass was then placed in an oven at 100 °C for 24 h until reaching constant weight [30].

2.6.2. Quantification of Lactic Acid and Residual Sugar

The quantification of lactic acid was determined in a high-performance liquid chromatograph (HPLC, Shimadzu, Kyoto, Japan) equipped with an ultraviolet detector at 210 nm. The Rezex ROA column (300 × 7.8 mm) from Phenomenex was used, eluted with 5 mM H₂SO₄ as the mobile phase at a flow rate of 0.6 mL/min and a temperature of 65 °C [30]. Samples were filtered through 0.22 µm membranes before injection. Identification and quantification were based on calibration curves of external standards of L-(+)-lactic acid and D-(+)-Xylose (Sigma-Aldrich, Darmstadt, Germany). The consumption of the carbon source was also determined in the HPLC, as described above, but equipped with a refractive index detector (RID) at 210 nm [30].

2.6.3. Statistical Analyses

The statistical analysis of the data related to the experimental design was carried out using the Statistica 7 software, employing multiple regression analysis by the method of least squares for each of the responses. The analysis considered the isolated terms, interactions, and quadratic terms of the variables studied as parameters.

2.6.4. Validation of Optimal Point and Fermentation Kinetics in Shaker

The experiments to validate the optimal point and fermentation kinetics in the shaker occurred at 150 rpm and 50 °C for 72 h.

3. Results and Discussion

3.1. Isolation and Selection

All colonies grown in the dish exhibited halos (Figure 1). However, 14 isolates were selected due to their good growth and larger halos resulting from the neutralization reaction of calcium carbonate with the organic acids produced. These isolates were identified as potential lactic acid producers and were investigated for lactic acid production. The results indicated that all were capable of producing the metabolite of interest. For the studies, eight isolates with the highest lactic acid production were chosen: BMI, BMAI, BLMI, B2L1, BUGC, B07, B03, and BUSI, with production levels ranging from 4.8 g·L⁻¹ to 9.9 g·L⁻¹.

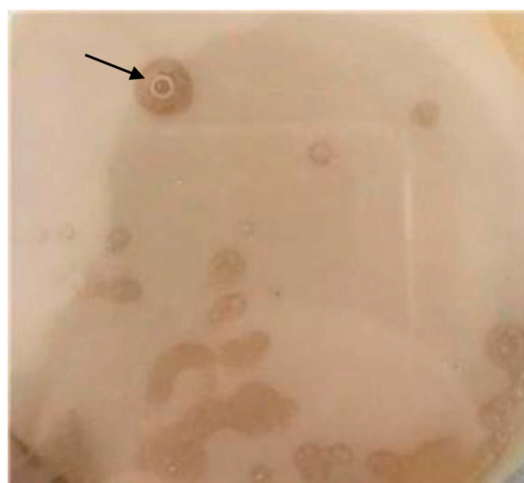


Figure 1. Bacteria grown in a Petri dish with XYP medium with the addition of calcium carbonate, after 48 h of incubation. Colony growth can be observed from the formation of the halo. The black arrow indicates a representation of the halo formation.

3.2. Biochemical Characterization and Molecular Identification

Gram staining revealed that the eight isolates were Gram-positive bacilli. Molecular identification based on 16S rRNA gene sequencing, a widely used method for classifying microorganisms, including members of the genus *Weizmannia*, placed all isolates within the *W. coagulans* clade. This analysis identified two species: *W. coagulans* and *W. ginsengihumi* (Table 2). The 16S rRNA gene has been extensively used in the isolation, identification, and taxonomic classification of *Weizmannia* spp., particularly in studies focusing on their probiotic potential [31–35].

Sequence analysis revealed that two groups of isolates (BMI/ B03 and BMAI/ BUGC/ BUSI) showed identical or highly similar 16S rRNA sequences when compared with GenBank data, indicating that they likely belong to the same clonal lineage. To avoid experimental redundancy and allow for comparative analysis between genetically distinct strains, only one representative isolate from each clonal group was selected for subse-

quent experiments. Thus, five non-clonal strains were chosen for the physiological and fermentative evaluations presented in the following sections.

Table 2. Identification of isolates according to the sequence obtained from the 16S rDNA region and comparison with GenBank data.

Strain	Accession Number	Coverage (%)	Percentage of Similarity (%)	Scientific Name
BMI	KF600778.1	95	97.46	<i>Weizmannia ginsengihumi</i>
BMAI	CP058594.1	99	96.81	<i>Weizmannia coagulans</i>
BLMI	AB680332.1	96	97.49	<i>Weizmannia coagulans</i>
B2L1	MT538507.1	96	97.49	<i>Weizmannia coagulans</i>
BUGC	CP058594.1	95	97.64	<i>Weizmannia coagulans</i>
B07	CP033687.1	98	97.27	<i>Weizmannia coagulans</i>
B03	KF600778.1	96	97.64	<i>Weizmannia ginsengihumi</i>
BUSI	CP058594.1	96	97.92	<i>Weizmannia coagulans</i>

3.3. Growth Curve

A growth curve experiment was performed to characterize the growth pattern of two isolated *Weizmannia* species, with a focus on determining the duration of the exponential phase. This information is essential for optimizing subsequent fermentation experiments, allowing cell transfers to be carried out at the ideal time—when cells are metabolically active and undergoing continuous division, minimizing cell death. *W. coagulans* BMAI and *W. ginsengihumi* BMI were chosen to represent their respective species. The exponential growth phase for both species began two hours and 30 min post-inoculation. After seven hours, the BMI culture was at the end of the exponential phase and entering the stationary phase, whereas BMAI reached the end of the exponential phase only after 10 h of cultivation (Figure 2). Subsequently, this experimental result was used to determine when these species are in the exponential growth phase, enabling the transfer of the pre-inoculum to the production medium during the period of active cell division. Based on these results, the microorganisms were always inoculated during their exponential growth phase, after 6 h of cultivation.

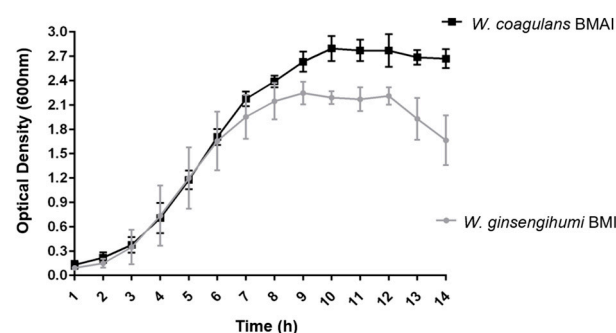


Figure 2. Growth curve of *W. coagulans* BMAI and *W. ginsengihumi* BMI in XYP medium at 50 °C and 150 rpm over 14 h of cultivation. Experiment conducted in triplicate.

This growth profile is consistent with observations by Olszewska-Widdrat et al. [36], who worked with *Weizmannia coagulans* cultivated in 10% yeast extract concentrations. Based on these results, the microorganisms were always inoculated during the exponential phase, specifically after six hours of growth.

3.4. Fermentation

Most strains demonstrated a peak in growth at 24 h. The BMAI strain apparently required a longer adaptation period to the medium and xylose metabolism, as evidenced by

the significant changes in consumption, growth, and lactic acid production starting at 12 h of fermentation. This strain also had the highest growth at 48 h, reaching $2.46 \pm 0 \text{ g} \cdot \text{L}^{-1}$. Conversely, the lowest growth was found in the *W. ginsengihumi* BMI isolate ($1.36 \pm 0 \text{ g} \cdot \text{L}^{-1}$) (Figure 3). The carbon source consumption profile was very similar for most isolates. Xylose was nearly depleted from the medium within 12 h of fermentation. The ability of *W. coagulans* to metabolize xylose rapidly is well documented. In fermentations involving this strain, xylose is almost entirely consumed within 12 h, resulting in low residual sugar, which is crucial for metabolite production [10,37–40].

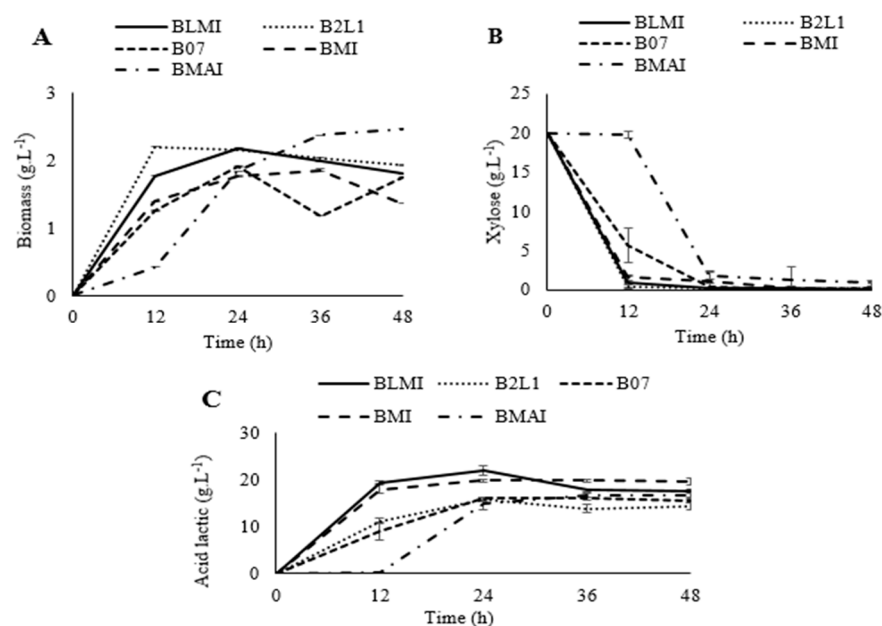


Figure 3. Growth profile (A), xylose consumption (B), and lactic acid production (C) by *Weizmannia* sp. isolates in XYP medium containing $20 \text{ g} \cdot \text{L}^{-1}$ of xylose in an anaerobic atmosphere. Cultivation occurred at 150 rpm and 50°C for 48 h of fermentation. The results obtained were the mean of three experiments.

Lactic acid production was influenced by the metabolism of the carbon source, with the highest levels observed between 12 and 24 h of fermentation by *W. coagulans* BLMI and *W. ginsengihumi* BMI, reaching $21.93 \pm 0.9 \text{ g} \cdot \text{L}^{-1}$ and $19.79 \pm 0.3 \text{ g} \cdot \text{L}^{-1}$ after 24 h, respectively. Previous studies have shown that *W. coagulans* can produce substantial amounts of lactic acid, primarily due to its ability to fully metabolize the available carbon source. In particular, the *W. coagulans* C106 strain demonstrated high xylose isomerase activity—an enzyme responsible for converting xylose into xylulose, a key step in xylose catabolism [41]. Xylulose is subsequently phosphorylated by xylulose kinase to form xylulose-5-phosphate, which enters either the pentose phosphate pathway or the phosphoketolase pathway, contributing to efficient lactic acid biosynthesis [42,43].

3.5. Experimental Design

The experimental design was conducted with the *W. ginsengihumi* BMI strain, as there is no known literature on lactic acid production using this species. The independent variables (carbon source, nitrogen source, and salt) and responses (lactic acid production, biomass, and residual sugar) are listed in Table 3. The results correspond to 48 h of fermentation, which was the time at which maximum organic acid production was reached in most trials.

Table 3. Central composite rotatable design employed in the cultivation of *W. ginsengihumi* BMI. The media were composed of xylose as the carbon source, yeast extract and peptone as nitrogen sources, and sodium acetate. The independent variables are represented by the actual levels. The responses correspond to 48 h of fermentation.

Trials	Variables (g·L ⁻¹)			Responses (g·L ⁻¹)			Productivity (g/L/h)	Product-to-Biomass Conversion (g/g)
	X ₁	X ₂	X ₃	Y ₁	Y ₂	Y ₃		
	Xylose	Nitrogen	Sodium Acetate	Lactic Acid	Biomass	Residual Sugar		
1	15.0	1.50	5.00	14.96	0.69	0.10	0.31	21.68
2	15.0	1.50	15.00	16.36	0.61	2.96	0.34	26.82
3	15.0	4.50	5.00	17.18	0.61	0.22	0.36	28.16
4	15.0	4.50	15.00	16.66	0.55	0.12	0.35	30.29
5	50.0	1.50	5.00	19.84	0.66	34.64	0.41	30.06
6	50.0	1.50	15.00	19.56	0.73	37.10	0.41	26.79
7	50.0	4.50	5.00	21.92	0.79	30.32	0.46	27.75
8	50.0	4.50	15.00	21.64	0.65	30.70	0.45	33.29
9	32.5	3.00	10.00	23.02	0.81	11.86	0.48	28.42
10	32.5	3.00	10.00	22.94	0.92	13.36	0.48	24.93
11	1.19	3.00	10.00	2.50	0.67	0.04	0.05	3.73
12	63.8	3.00	10.00	23.38	1.09	37.76	0.49	21.45
13	32.5	0.31	10.00	0.34	0.17	32.90	0.01	2.00
14	32.5	5.68	10.00	22.76	1.01	11.22	0.47	22.53
15	32.5	3.00	1.050	22.34	1.28	12.46	0.47	17.45
16	32.5	3.00	18.94	25.28	1.30	8.38	0.53	19.45
17	32.5	3.00	10.00	24.22	1.28	9.16	0.50	18.92
18	32.5	3.00	10.00	25.20	1.20	6.34	0.53	21.00

At this sampling point, lactic acid production ranged from 0.34 g·L⁻¹ to 25.20 g·L⁻¹ (Trials 13 and 16, respectively). These same trials had the lowest and highest bacterial growth in dry mass (0.17 g·L⁻¹ and 1.30 g·L⁻¹, respectively). It is noteworthy that Trial 13 had the lowest nitrogen source concentration, which is an essential factor for microorganism growth. Carbon and nitrogen sources are expected to influence bacterial growth, as these are essential nutrients for cellular composition and growth energy, especially yeast extract due to its high content of nitrogen compounds, purines, pyrimidines, and vitamins [44–46].

The trials with the best responses for Y₁ (lactic acid production) and Y₂ (biomass) did not correspond to the response Y₃ (residual sugar), as the lowest residual sugar concentrations were observed in trials conducted at coded levels of −1 (Trial 1) and −1.788 (Trial 11). Ouyang et al. [47] used 80 g·L⁻¹ of xylose as the carbon source for the *W. coagulans* NL01 strain, achieving 60.8 g·L⁻¹ of lactic acid and approximately 7.5 g·L⁻¹ of residual sugar. Using 100 g·L⁻¹ of xylose, lactic acid production reached 75 g·L⁻¹, but the amount of residual sugar increased to 15 g·L⁻¹. The maximum xylose concentration in the experimental design was 63.80 g·L⁻¹, resulting in 37.76 g·L⁻¹ of residual xylose. However, this concentration did not yield the highest lactic acid production or biomass (Trial 12). Therefore, higher xylose concentrations may be causing growth inhibition and consequently decreasing lactic acid production.

Despite varying at coded levels of 0 and +1.788 in these trials, the variable X₃ (sodium acetate) did not influence the responses in the central composite design. The lack of significance of sodium acetate can be seen in Trials 15 and 16, which have very similar responses but are both alpha points of the matrix.

Based on the responses for lactic acid production (Y₁), biomass (Y₂), and xylose consumption (Y₃), multiple regression analyses were performed, resulting in the regression equations (Equation (2)) presented below:

$$Y_1 = 23.95 + 3.82 (X_1) + 3.24 (X_2) + 0.38 (X_3) - 3.09 (X_1^2) - 3.53 (X_2^2) + 0.30 (X_3^2) + 0.20 (X_1X_2) - 0.18 (X_1X_3) - 0.24 (X_2X_3)$$

$$Y_2 = 1.25 + 0.03 (X_1) + 0.04 (X_2) + 0.08 (X_3) - 0.14 (X_1^2) + 0.02 (X_2^2) + 0 (X_3^2) - 0.14 (X_1X_2) - 0.23 (X_1X_3) - 0.02 (X_2X_3) \quad (2)$$

$$Y_3 = 10.19 + 13.66 (X_1) - 3.62 (X_2) - 0.11 (X_3) - 2.77 (X_1^2) + 3.76 (X_2^2) + 0.12 (X_3^2) - 1 (X_1X_2) + 0.01 (X_1X_3) - 0.63 (X_2X_3)$$

The regression equation (Equation (2)) enabled the generation of the response surface plots (Figure 4), which illustrate the effects of the interactions among significant variables and identify the optimal levels of each. The aim was to identify the best cultivation conditions for achieving maximum lactic acid production by the BMI strain.

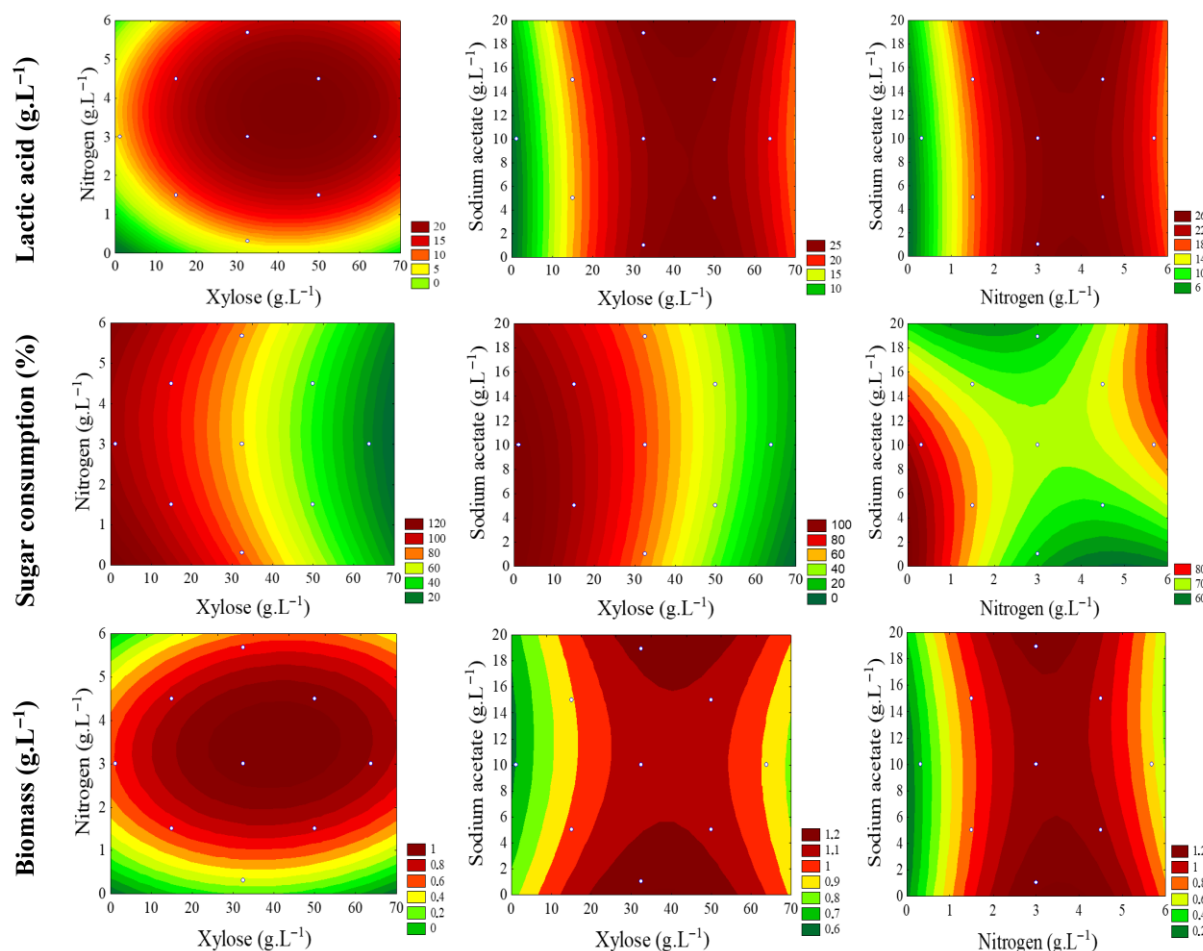


Figure 4. Contour curve for lactic acid production, sugar consumption, and biomass by *W. ginsengihumi* BMI as a function of carbon and nitrogen sources.

Concentrations of 30 to 55 g·L⁻¹ of xylose and 2.6 to 4.9 g·L⁻¹ of the nitrogen source (highlighted in red zone of graph) yielded the highest concentrations of the metabolite of interest and also supported the most significant microbial growth (Figure 3).

The species *W. coagulans*, which belongs to the same clade as *W. ginsengihumi*, has been reported in the literature to exhibit promising characteristics for lactic acid production using glucose as a carbon source. However, several challenges are associated with lactic acid production from pentoses, such as xylose [38]. In studies by Wang et al. [48], the *W. coagulans* XZL9 strain was capable of xylose consumption, but only when the concentration of the carbon source was below 70 g·L⁻¹. Concentrations of xylose above 100 g·L⁻¹ resulted in lower lactic acid concentrations and higher residual xylose.

According to the response surface graph (Figure 4), the BMI strain will show a decline in lactic acid production if the carbon source concentration exceeds 70 g·L⁻¹. Ye et al. [41]

found a reduction in xylose isomerase and lactate dehydrogenase activities in strain *B. coagulans* C106 at higher substrate concentrations, suggesting that substrate inhibition is primarily due to the decreased rate of substrate entry into the cells, although the detailed mechanism remains unclear.

As a relatively new species [49] with no current records correlating it as a lactic acid producer, it is of interest to modify this strain to enhance its tolerance to high xylose concentrations. Alternatively, substrate inhibition could be addressed through fed-batch fermentation [50]. Zheng et al. [38] used atmospheric and room temperature plasma (ARTP) mutagenesis to overcome inhibition issues related to high xylose concentrations. This approach enabled the NL-CC-17 strain of *W. coagulans* to produce $90.29 \text{ g}\cdot\text{L}^{-1}$ of lactic acid while fermenting $100 \text{ g}\cdot\text{L}^{-1}$ of xylose.

Productivity results did not vary significantly and exhibited relatively small dispersion (mean = 0.394 g/L/h ; variance = 0.022 g/L/h). Correlations with the response analyzed were found for X_1 (0.501) and X_2 (0.422). The highest values were found in Trials 16 and 18, with productivity of 0.53 g/L/h . In both trials, the xylose and nitrogen concentrations were 32.5 and $3.0 \text{ g}\cdot\text{L}^{-1}$, respectively. Regarding the conversion of product to biomass, correlations between this response and variables X_1 (0.325) and X_2 (0.388) suggest that higher concentrations of the carbon and nitrogen sources positively influenced product-to-biomass conversion. However, X_3 was not significantly correlated with the response analyzed (0.1). The trials with the highest product-to-biomass conversions were Trial 8, with conversion of 33.29 g/g , and Trial 4, with conversion of 30.29 g/g , with nitrogen concentrations of $4.5 \text{ g}\cdot\text{L}^{-1}$ in both cases and xylose concentrations of 50 and $15 \text{ g}\cdot\text{L}^{-1}$, respectively.

In Trial 4 of the central composite design, the use of a lower xylose concentration ($15 \text{ g}\cdot\text{L}^{-1}$) resulted in a residual sugar content of only $0.12 \text{ g}\cdot\text{L}^{-1}$, indicating nearly complete substrate consumption. Although lactic acid production was $16.66 \text{ g}\cdot\text{L}^{-1}$, the overall yield and productivity were lower than those observed in Trial 16. Under optimized conditions, Trial 16 achieved $25.28 \text{ g}\cdot\text{L}^{-1}$ of lactic acid with higher productivity ($0.53 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$) and efficient substrate utilization, despite a residual sugar level of $8.38 \text{ g}\cdot\text{L}^{-1}$. This demonstrates that complete sugar consumption is not strictly necessary to obtain high lactic acid yields and that Trial 16 offers a better balance between productivity and efficiency for industrial applications.

Additionally, when compared to the fermentation in Figure 3, where lactic acid production reached $19.79 \pm 0.3 \text{ g}\cdot\text{L}^{-1}$ after 24 h using $20 \text{ g}\cdot\text{L}^{-1}$ of xylose and $15 \text{ g}\cdot\text{L}^{-1}$ of nitrogen, the high nitrogen level likely stimulated faster growth and product formation. However, that experiment was not optimized and did not explore parameter variations. In the central composite design, fermentation was evaluated at 48 h, as most trials peaked within this period.

Thus, it can be inferred that among the variables studied, xylose and nitrogen are the primary factors affecting both conversion and productivity, with xylose exerting a more significant impact on productivity. For optimization of the process, high xylose levels should be maintained, but nitrogen levels should be regulated to direct the metabolism of the microorganism towards lactic acid production rather than merely supporting growth. Conversely, sodium acetate has a minimal impact on both parameters. This compound may act as an additional carbon source or buffer, but does not appear to play a significant role in regulating the main metabolic pathway for lactic acid production. Under certain conditions, acetate may even inhibit fermentation by accumulating to toxic levels.

3.6. Validation of Optimal Point and Fermentation Kinetics in Shaker

Based on the previously presented results and considering that optimized fermentation should achieve the maximum lactic acid concentration and the lowest possible residual

sugar concentration, the chosen parameters were $32.5 \text{ g}\cdot\text{L}^{-1}$ of xylose and $3.0 \text{ g}\cdot\text{L}^{-1}$ of the nitrogen source (Trial 16). As sodium acetate did not influence the responses of interest (metabolite produced and bacterial growth) (Figure 4), it was excluded from the fermentation medium.

A fermentation kinetics study was then conducted to observe the behavior of the BMI microorganism with regard to lactic acid production, bacterial growth in biomass formation (dry weight), and xylose consumption over time (Figure 5). Fermentation was carried out for 72 h to determine whether growth and production would continue over time and consequently reduce the concentration of residual xylose. As shown in Figure 5, lactic acid production entered the exponential phase after 12 h, reaching a maximum concentration of $20.20 \text{ g}\cdot\text{L}^{-1}$ at 72 h—the highest value observed. However, at 36 h, production had already reached $20.02 \text{ g}\cdot\text{L}^{-1}$, with a higher productivity of $0.55 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ compared to $0.28 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ at 72 h, and a residual sugar concentration of $12.59 \text{ g}\cdot\text{L}^{-1}$. These results suggest that most of the production occurred before 36 h.

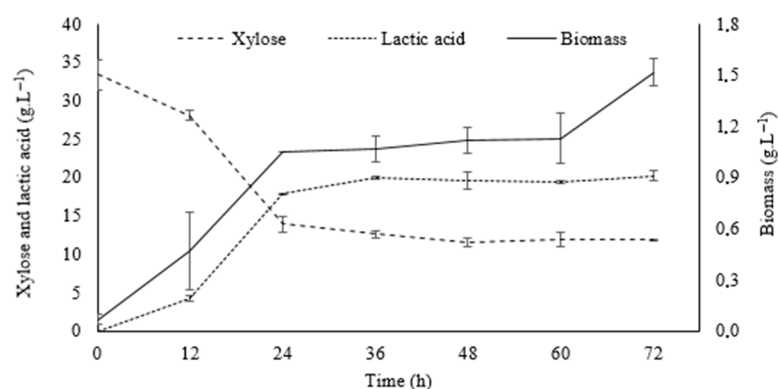


Figure 5. Kinetics of lactic acid production, xylose consumption, and microbial growth by *W. ginsengihumi* BMI.

The highest instantaneous lactic acid production rate ($1.13 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$) and xylose consumption rate ($1.18 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$) both occurred between 12 and 24 h, indicating that substrate utilization was closely coupled with metabolite synthesis during this phase.

The growth curve also revealed that the highest biomass concentration ($1.5 \text{ g}\cdot\text{L}^{-1}$) was achieved at 72 h. These findings suggest that extending the fermentation period beyond 72 h might promote further xylose consumption and possibly a modest increase in lactic acid production. However, the production plateau observed at 36 h—along with the peak productivity and substrate consumption in the 12–24 h interval—aligns with the trends previously observed in batch fermentation (Figure 3), emphasizing that this period is likely the most efficient phase of the process.

4. Conclusions

This study focused on the production of lactic acid from xylose by a novel isolate of *Weizmannia ginsengihumi*. No bibliographic records were found of this species producing this biomolecule. The experimental design revealed that sodium acetate, which is a component of the XYP medium, is not necessary for growth or lactic acid production. However, the carbon source (xylose) and nitrogen source (yeast extract and peptone) were crucial factors for obtaining lactic acid.

Further studies should be conducted with different cultivation media using this species and exploring alternative carbon sources. Given that the BMI strain has shown potential as a lactic acid producer, continuing research on this species of the coagulans clade is important. Other critical factors for lactic acid production, such as the use of waste products, should

also be considered. This is the first time a strain of *W. ginsengihumi* has been described as a lactic acid producer.

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